ORIGINAL

FORM 2

THE PATENTS ACT 1970

(39 of 1970)

AND

The Patents Rules, 2003

COMPLETE SPECIFICATION

(See section 10 and rule13)

1. TITLE OF THE INVENTION:

"PROCESS FOR PREPARATION OF DL-HOMOCYSTEINE THIOLACTONE HYDROCHLORIDE"

2. APPLICANT (S):

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3. PREAMBLE TO THE DESCRIPTION:

The following specification particularly describes the invention and the manner in which it is to be performed.

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Technical Field of Invention:

The present invention relates to a process for preparation of DL-Homocysteine thiolactone hydrochloride having inorganic salt impurity less than 0.1% and HPLC purity more than 99.8%.

Background of the invention:

Amino-3 dihydrothiophenon-2 or Homocysteine thiolactone, hereinafter called HTL, was described by Du Vigneaud in 1935. It was prepared by dealkylation of DL-Methionine or another alcoyl homocysteine by reduction by sodium in ammonia or hydroiodic acid, followed by cyclization.

The physiological and therapeutic importance of amino acids bearing sulphhydryl groups is known. For example, the use of cysteine hydrochloride in the treatment of liver diseases has been well proved. However these amino acids is unstable and even the action of atmospheric oxygen causes it to be converted at least partially into the physiologically ineffective cysteine. Due to its acid reaction, physiological difficulties exist in the use of cysteine hydrochloride.

It has now been found that homocysteine thiolactone can be used with excellent results in place of cysteine hydrochloride. In the weakly alkaline blood medium, this thiolactone is split to give homocysteine.

Also, Homocysteine thiolactone is a key raw material used for Erdosteine (Mucolytic), Citiolone (hepatic disorders), N-Butyryl-DL-homocysteine thiolactone, 2-chloro-N-(tetrahydro-2-oxo-3-thienyl)-3-Pyridinecarboxamide and homocystamide L-lysine adduct.

In another use, N-acyl DL-homocysteine thiolactones when added to a light-sensitive silver halide emulsion is observed to stabilize and inhibit the fogging of the emulsion. US3068100 describes DL-Homocysteine thiolactone and its other derivatives such as N-butyryl DL-homocysteine thiolactone and N-propionyl DL-homocysteine thiolactone which are used as stabilizing and antifogging agents.

Literature on the processes for the preparation of Homocysteine thiolactone is given below:

Journal of Biological Chemistry 30 (1939) 305; 112 (1935) 149 and Justus Liebigs Annalen der Chemie 599 (1956) 23 describes preparation of HTL by treating homocysteine with hydrochloric acid and tin foil. However, use of tin metal is not feasible for commercial production due to its cost and its removal from the product.
Journal of Biological Chemistry; vol. 106; (1934); 451 and vol. 234; (1959); p. 516 describes preparation of HTL by using aqueous hydriodic acid, hypophosphoric acid which is however not commercially feasible.

GB831844 discloses preparation of homocysteine thiolactone from homocysteine by reduction with tin or aluminium in the presence of sulphuric acid. The homocysteine is formed by demethylation of methionine in acid solution. The total yield, based on the methionine which is used, is not more than 35% of the theoretical yield. Further, the isolation of homocysteine thiolactone obtained by this process presents great difficulties owing to the large quantity of salt which is formed by the neutralization of the strongly acid solutions.

Further, the said patent describes the formation of homocysteine thiolactone wherein, DL-methionine is initially demethylated in presence of a solvent by sodium metal in liquid ammonia. The excess sodium is removed by adding ammonium chloride and then the liquid ammonia is distilled off. The homocysteine obtained is dissolved in methanol and acidified with concentrated hydrochloric acid. The precipitated sodium chloride is filtered. The filtrate is evaporated to dryness in vacuo under CO2. The residue is dissolved in and recrystallized from ethyl alcohol. The yield is 75%. This patent however does not describe assay and content of inorganic material present after recrystallization of HTL. Further, removal of the side product, sodium chloride in aqueous medium or ethyl alcohol is very difficult as both are soluble in water.

DE2547672 describes preparation of HTL by demethylation of DL-methionine by using sodium metal in liquid ammonia at pressure 8 to 9 atm and temperature 8°C, followed by cyclization by using hydrochloric acid. The sodium chloride salt is then removed using mixture of IPA and water at 70°C. The method gives low yield and is contaminated with inorganic material.

US4072703 describes new industrial products, in the free form or in the form of basic or acidic organic and inorganic salts of the new HTL derivatives which includes the characteristic grouping –S-CH-CH_2-CH(NHR)-CO, where R is hydrogen, C_1-C_4 acyl group.

In view of the non-effective removal of undesired inorganic salt form the final product as observed in the above prior arts, it is an object of the present invention to develop an efficient and simpler process for preparation of Homocysteine thiolactone acid addition salt with high purity and in satisfactory yield.

The further object of the present invention is an efficient removal of sodium chloride impurity from the product using phase transfer catalyst.

Summary of invention:

The present invention discloses preparation of DL-Homocysteine thiolactone hydrochloride from DL-methionine. In an aspect, disodium salt of DL-homocysteine is obtained from DL-methionine by treatment with sodium in liquid ammonia followed by cyclisation to obtain DL-Homocysteine thiolactone hydrochloride.

In an aspect, during conversion of DL-methionine to DL-disodium methionine the excess sodium is decomposed by C1-C4 alcohol to obtain white solid material consisting of DL-Homocysteine, sodium methoxide and sodium amide. This mixture is then treated with DM water at 25-30°C under nitrogen atmosphere, wherein, the solid dissolves to give a clear solution (ammonia gas formed is distilled off to maintain pH of the reaction).

In a further aspect, the clear solution is reacted with conc. HCl at reflux temperature wherein cyclisation of DL-methionine to DL-Homocysteine thiolactone hydrochloride takes place. Water and hydrochloric acid are distilled off under vacuum till water remains 50 – 70 %.

In a preferred aspect, after cyclisation reaction, the excess water and hydrochloric acid is removed by azeotropic distillation in presence of aromatic hydrocarbon or its derivatives at 100-110°C to obtain water free DL-homocysteine thiolactone hydrochloride which is further isolated by dissolving in C1-C4 alcohol.

In another preferred aspect of the present invention, sodium chloride salt obtained as impurity during cyclisation process is removed using phase transfer catalyst in variable percentage of aqueous medium.

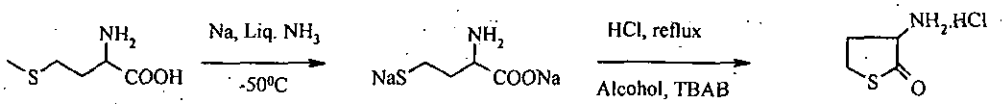
In yet another aspect, sodium chloride can be removed by employing higher homolog of alcohol such as ethanol, butanol and IPA in presence or absence of aqueous medium.

DL-Homocysteine thiolactone hydrochloride thus obtained has sodium chloride less than 0.1% with HPLC purity more than 99.8%.

Detailed description of invention:

The present invention describes a process for preparation of DL-Homocysteine thiolactone hydrochloride from DL-methionine in good yield and high purity.

In an embodiment, disodium salt of DL-homocysteine is obtained from DL-methionine by treatment with sodium in liquid ammonia at – 50 to -60°C. DL-homocysteine thus obtained is then subjected to cyclisation to obtain the final product by acidification in presence of solvent. The process scheme is given below:



DL-Methionine | Isolated as crude | DL-Homocysteinethiolactone hydrochloride

In an embodiment, the excess sodium remained during the conversion of DL-methionine to DL-disodium methionine is decomposed using C1 – C4 alcohol by maintaining the reaction mass temperature -50 to -45°C under nitrogen atmosphere followed by removal of ammonia. The white solid material formed after ammonia removal contains disodium salt of DL-Homocysteine, sodium methoxide and sodium amide. To the said solid material is then added DM water at 25-30°C, wherein the solid dissolves to give a clear solution free of ammonia gas.

In a preferred embodiment, after cyclisation of DL-disodium methionine to DL-Homocysteine thiolactone hydrochloride (HTL), the excess water is removed from the reaction mixture by azeotropic distillation in presence of aromatic hydrocarbon or its

derivatives at 100-110°C to obtain water free DL-homocysteine thiolactone hydrochloride which is further isolated by dissolving in C1-C4 alcohol.

In another preferred embodiment of the present invention, the key to achieving high purity of DL-Homocysteine thiolactone hydrochloride lies in the effective removal of sodium chloride formed during cyclisation reaction. This is achieved by employing a phase-transfer catalyst. Suitable phase-transfer catalyst includes, for example, quaternary ammonium salts, phosphonium salts such as Tetrabutylammonium bromide (TBAB), Hexadecyltrimethylammonium bromide, Tetraethylammonium chloride hydrate, Tributylhexadecylphosphonium bromide, Tetrabutylphosphonium chloride, Tetrahexylammonium hydrogensulfate, Tetrabutylammonium hydroxide, Tetraphenylphosphonium chloride, Tetrabutylammonium nonafluorobutanesulfonate, Tetrabutylammonium heptadecafluorooctanesulfonate and Tetraethylammonium chloride hydrate. Tetrabutylammonium bromide (TBAB) is most preferred. TBAB acts as lowering of surface tension agent and as well as form intermolecular bonding with ionic solvent. Due to this property TBAB helps in decreasing solubility of sodium chloride by formation of salt with intermolecular bonding with sodium chloride, thus facilitating easy removal of the undesired compound and enhancing the purity and yield of the final product.

Accordingly, sodium chloride is removed by using phase transfer catalyst in variable percentage of aqueous alcoholic solution.

In another embodiment, removal of inorganic salt is carried out employing higher homolog of alcohol such as ethanol, butanol and IPA in presence or absence of aqueous medium.

DL-Homocysteine thiolactone hydrochloride thus obtained has sodium chloride content less than 0.1% with HPLC purity more than 99.8%.

The process of the present invention is further described in detail as follows:

In an aspect of the present invention, DL-methionine is treated with sodium metal in liquid ammonia (obtained by passing ammonia gas through calcium oxide traps in ammonia condenser) at a temperature between -50 to -60°C for 2-3 hours. The blue color

(sodium dissolved in ammonia) is maintained for 1 to3 hours at -50° to -45°C under nitrogen atmosphere. This is followed by addition of a solvent selected from C1-C4 alcohol to decompose excess of sodium metal at -50° to -45°C and under nitrogen atmosphere (dark blue color become light blue color and finally white after complete conversion of DL-methionine to DL-disodium methionine). The temperature of the reaction mass is then raised to 25 to 30°C under nitrogen atmosphere to collect liquid ammonia through 'ammonia cooling condenser' which can be used for next batch experiment. The white solid material formed after ammonia removal contains disodium salt of DL-homocysteine, sodium methoxide and sodium amide.

To the said solid material is then added DM water at 25-30°C under nitrogen atmosphere to obtain clear solution, wherein, the ammonia formed is removed by distillation to maintain the pH of reaction mass neutral. This is followed by the cyclisation of disodium salt of DL-homocysteine to DL-homocysteine thiolactone by addition of mineral acid such as hydrochloric acid and heated to reflux. Distilled out water and hydrochloric acid under vacuum till water remains 50 – 70 % and remaining water and hydrochloric acid is removed by azeotropic distillation in presence of aromatic hydrocarbon or its derivatives at 100-110°C to obtain water free DL-homocysteine thiolactone hydrochloride.

Cyclisation of disodium salt of DL-homocysteine to DL-homocysteine thiolactone hydrochloride with an acid results in the formation of sodium chloride which has an adverse effect on the purity of DL-homocysteine thiolactone hydrochloride. The residue obtained is then dissolved in C1-C4 alcohol, heated to 60-70°C, filtered to remove sodium chloride followed by addition of catalytic amount of phase transfer catalyst. The reaction mass is cooled to 5-10°C, filtered and further washed with chilled C1-C4alcohol and dried under vacuum. Sodium chloride content was observed to be in the range of 0.5 – 10.0 % and yield on the basis of DL-Homocysteine thiolactone hydrochloride is 80%.

In another aspect, crude HTL is dissolved in 1 – 40% of aqueous C1-C4 alcohol followed by addition of tetrabutylammonium bromide (TBAB) in catalytic amount. The reaction mass is heated to 50-60°C. Sodium chloride precipitated out with TBAB is filtered at 40°C. The filtrate is again heated to 50-60°C by addition of activated carbon and filtered the reaction mass. Cooled the reaction mass to 5-10°C. Filtered the product and washed with chilled alcohol (C1 –C4) with or without 1 - 40% aqueous medium, dried under

vacuum to obtain pure HTL of pharma grade having sodium chloride less than 0.1% and HPLC purity more than 99.8% with DL-Homocysteine and 4,4'-Disulphanediylbis(2-aminobutanoic acid) less than 0.1%.

Further details of the process of the present invention will be apparent from the examples presented below. Examples presented are purely illustrative and are not limited to the particular embodiments illustrated herein but include the permutations, which are obvious as set forth in the description.

Examples:

Example1

Preparation of DL- Homocysteine Thiolactone Hydrochloride

To DL-Methionine (1.0 mole) was added 14 volume liquid ammonia (obtained by passing ammonia gas through calcium oxide traps in a line to ammonia condenser) at -50 to -60°C. This was followed by addition of small pieces of sodium metal (3.1 mol) in lots in 2 - 3 hrs at -50to -60°C(after 1.0 mole sodium metal addition, blue color appears and after less than 5 min it disappears and color of solution become white; after 2.3 mole blue color persist 5 – 10 min). The blue color (sodium dissolved in ammonia) is maintained for 1 to3 hours at -50 to -45°C under nitrogen atmosphere. Methanol (1.0 mole) was then added slowly within 1.0 hr to decompose excess of sodium metal maintaining the temperature at -50to -45°C under nitrogen atmosphere (dark blue color become light blue color and finally white). Removed the ice cooling bath and raised the temperature of reaction mass to 25° to 30°C under nitrogen atmosphere to collect liq. ammonia through 'ammonia cooling condenser' for next batch experiment. The white solid material formed after ammonia removal contains disodium salt of DL-Homocysteine, sodium methoxide and sodium amide. Added deoxygenated DM Water (6.5 volume) at 25°to 30°C without stirring under nitrogen atmosphere, solid dissolved to get clear solution with evolution of ammonia gas. [Sodium amide react with water, liberates ammonia gas (which is collected and reused) and generates sodium hydroxide]. Distilled off water (2 to 3.5 volumes) to remove traces of ammonia by confirming pH of reaction mass neutral. Added concentrated hydrochloric acid (4-6 mole) slowly. Heated to reflux and maintained for 2.0–5 hrs, distilled out water upto 1.0 volume during maintaining of refluxing. Distilled out water and hydrochloric acid by azeotropic distillation at 100-110°C with 2 volume

toluene, to get reaction mass water free. Distilled off toluene upto 80% and remaining toluene is stripped with methanol. Added 3 volume methanol and heated to 40-50°C and stirred the reaction mass for 1.0 hr. Filtered reaction mass to remove sodium chloride. Distilled off methanol and added 3 volume IPA. Cooled the reaction mass to 5-10°C and maintained for 1.0 hr, filtered the product and further washed with chilled IPA. Dried under vacuum to yield 80% HTL containing sodium chloride in the range of 0.8 to 10 %. and HPLC purity more than 99.0%

Example2

Preparation of DL- Homocysteine thiolactone Hydrochloride

To DL-Methionine (1.0 mole) was added 14 volume liquid ammonia (obtained by passing ammonia gas through calcium oxide traps in a line to ammonia condenser) at -50° to -60°C. This was followed by addition of small pieces of sodium metal (3.1 mol) in lots in 2 - 3 hrs at -50° to -60°C (after 1.0 mole sodium metal addition, blue color appears and after less than 5 min it disappears and color of solution become white; after 2.3 mole blue color persist 5 – 10 min). The blue color (sodium dissolved in ammonia) is maintained for 1-3 hours at -50° to -45°C under nitrogen atmosphere after addition of 3.1 mole sodium metal.C1 – C4 alcohol (1.0 to 5 mole) was then added slowly within 1.0 hr to decompose excess of sodium metal by maintaining the reaction mass temperature -50 to -45°C under nitrogen atmosphere (dark blue color become light blue color and then white) after 1.0 h. Removed the ice cooling bath and raised the temperature of reaction mass to 25 - 30°C under nitrogen atmosphere to collect liq. ammonia through 'ammonia cooling condenser' for next batch experiment. The white solid material thus formed after ammonia removal contains disodium salt of DL-Homocysteine, sodium methoxide and sodium amide. Added deoxygenated DM Water (6.5 volume) at 25-30°C without stirring under nitrogen atmosphere, solid dissolved to get clear solution with evolution of ammonia gas. [sodium amide react with water, liberates ammonia gas (which is collected and reused) and generates sodium hydroxide]. Distilled off water (2 to 3.5 volumes) to remove traces of ammonia by confirming pH of reaction mass neutral. Added concentrated hydrochloric acid (4-6 mole) slowly. Heated the reaction mass to reflux and maintained for 2.0–5 hrs, distilled out water and hydrochloric acid under vacuum till water remains 50 – 70 % and remaining water by azeotropic distillation at 100-110°C with 2-5 volume toluene, to get reaction mass water free. Cooled the reaction mass to 35 – 40°C and filtered the product.

Suck dried the product and further dried under vacuum at 50-70°C for 10 hrs till LOD is less than 0.5%. The sodium chloride content is 50 – 60% Taken dried product and added 90% aqueous 5 volume IPA and heated to 60-70°C and stirred the reaction mass for 1.0 hr. Filtered reaction mass to remove sodium chloride. Added activated carbon and catalytic amount of phase transfer catalyst, heated the reaction mass to 55– 65°C and maintained for 30 min, filtered the reaction mass through hyflo. Cooled the reaction mass to 5-10°C and maintained for 2-4 hr, filtered the product and further washed with chilled 1-40% aqueous IPA. Dried under vacuum to yield 80% HTL containing sodium chloride less than 0.1% and HPLC purity more than 99.7% with DL-Homocysteine and 4,4'-Disulphanediylbis(2-aminobutanoic acid) less than 0.1%.

Example 3

Purification of DL- Homocysteine thiolactone Hydrochloride

To crude HTL (example-1) was added 3-5 volumes of 0:5 to 20% aqueous IPA and tetrabutylammonium bromide (TBAB) in catalytic amount. Heated the reaction mass to 50-60°C. Sodium chloride precipitated out with TBAB was filtered out at 60-70°C. Cooled the reaction mass to 5-10°C and maintained for 3-5hrs. Filtered the product and further washed with chilled IPA with or without 0.5 to 20% aqueous medium, dried under vacuum to 85% yield of pure HTL of pharma grade having sodium chloride less than 0.1% and HPLC purity 99.7% with DL-Homocysteine and 4,4'-Disulphanediylbis(2-aminobutanoic acid) is less than 0.1%.

We Claim,

1. The process for preparation of DL-Homocysteine thiolactone hydrochloride with high purity comprising the steps of;
    a. Demethylating DL-methionine with sodium-liq NH_3 followed by addition of C1-C4 alcohol to obtain reaction mixture containing disodium salt of DL-homocysteine, sodium methoxide and sodium amide,
    b. treating the mixture of disodium salt of DL-Homocysteine, sodium methoxide and sodium amide of step (a) with DM water to get clear solution with simultaneous removal of ammonia,
    c. Treating the clear solution obtained in step (b) with concentrated hydrochloric acid at reflux to obtain DL-Homocysteine thiolactone hydrochloride,
    d. Distilling out excess water and hydrochloric acid from reaction mixture of DL-Homocysteine thiolactone hydrochloride obtained in step (c), using aromatic hydrocarbons or its derivatives at 100-110°C, and
    e. precipitating sodium chloride impurity formed in step (c) by heating with phase transfer catalyst in aqueous alcoholic solvent followed by cooling the filtrate to obtain DL-Homocysteine thiolactone hydrochloride
2. The process as claimed in claim 1, wherein the phase transfer catalyst in step (e) is selected from quaternary ammonium salts, phosphonium salts such as Tetrabutylammonium bromide (TBAB), Hexadecyltrimethylammonium bromide, Tetraethylammonium chloride hydrate, Tributylhexadecylphosphonium bromide, Tetrabutylphosphonium chloride, Tetrahexylammonium hydrogensulfate, Tetrabutylammonium hydroxide, Tetraphenylphosphonium chloride, Tetrabutylammonium nonafluorobutanesulfonate, Tetrabutylammonium heptadecafluorooctanesulfonate and Tetraethylammonium chloride hydrate.
3. The process as claimed in claim 1 & 2, wherein the phase transfer catalyst employed is Tetrabutylammonium bromide (TBAB).
4. The process as claimed in claim 1, wherein the alcohol for step (e) is selected from C1-C4.
5. The process as claimed in claim 1, wherein the percentage range of aqueous alcoholic solvent is 1-40%.

6. The process as claimed in claim 1, wherein aromatic hydrocarbons or its derivatives is selected from, benzene, toluene and xylene.
7. The process as claimed in claim 1, wherein sodium chloride content in DL-Homocysteine thiolactone hydrochloride is less than 0.1%

Dated this 22nd day of February, 2010

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